Communications to the Editor

Kauluamine: An Unprecedented Manzamine Dimer from an Indonesian Marine Sponge, *Prianos* sp.

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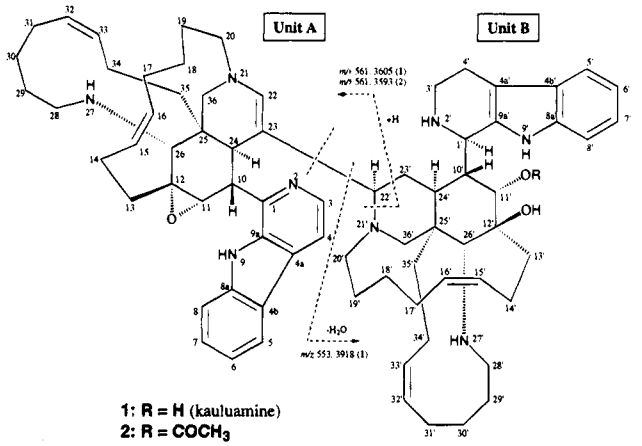
The manzamines, first described in 1986,[2] are an intriguing group of marine alkaloids, which are characterized by a fused and bridged tetra- or pentacyclic ring system that is joined to a β-carboline. As is usually the case with natural products, minor structural variants continue to be isolated.[3] The biogenesis of the manzamines was initially baffling, although the recent ingenious suggestion[4] of an intramolecular Diels–Alder reaction in a macrocyclic bisdihydropyridine, though not yet confirmed in the laboratory, is entirely plausible. We now describe the structure of kauluamine (**1**),[5] which was isolated from an Indonesian sponge *Prianos* sp.[6] It is an unsymmetrical manzamine dimer, thus adding yet another level of complexity to this fascinating group of alkaloids.

The sponge (dry weight 210 g) was collected in Manado Bay, Indonesia, by SCUBA and kept frozen until used. The dichloromethane–ethanol (5:1) soluble part of the ethanol extracts was separated by high-speed countercurrent chromatography (HSCCC) to yield kauluamine (**1**, 160 mg), $[\alpha]_D$ +0.7° (*c* 0,18, $CHCl_3$), as an unstable pale yellow solid.[7] A molecular formula was established by HR-FABMS as $C_{72}H_{94}N_8O_3$ (*m/z* 1101.7426 for $C_{72}H_{93}N_8O_2$ [MH − H_2O]$^+$, Δ −0.4 mmu).[8] In the DEPT spectrum 14 C, 29 CH, and 29 CH_2 signals were observed, accounting for all carbons. Failure of **1** to react with CH_2N_2 or to give a positive reaction with $FeCl_3$ on TLC indicated the absence of a carboxylic acid or of a phenol. The NMR spectral features of the manzamines[2c,3a] and a consideration of the molecular formula suggested a close relationship to the manzamines, perhaps of a dimeric nature. ^1H and ^{13}C NMR spectra in several solvents exhibited only broad signals at temperatures ranging from 25 to 85 °C.[9] The presence of a secondary hydroxyl group was confirmed by acetylation of **1** with pyridine and acetic anhydride. Acetate **2**,[10] $C_{74}H_{96}N_8O_4$ (FABMS *m/z* 1143.7510 for $C_{74}H_{95}N_8O_3$ [MH − H_2O]$^+$, Δ −1.4 mmu),[8,11] was much more stable toward air oxidation or acid than alcohol **1** and gave sharper NMR signals; therefore structure determination was carried out with **2**. ^1H and ^{13}C NMR signals (Bruker AMX600, 55 °C) of **2** (about 2 mg in 0.5 mL of CD_3CN) heavily overlapped, especially in the upfield region (63 protons at δ 1–3 and 28 carbons at δ 19–40). Suitable crystals for X-ray study could not be obtained for **2** or **1**. Thus the structure of **2** was elucidated by NMR spectral methods, mainly on the basis of extensive 2D NMR experiments, such as DQFCOSY, HOHAHA, HMQC, HMBC, and HMQC-HOHAHA.



1: R = H (kauluamine)
2: R = $COCH_3$

Unit A. The aromatic region in the ^{13}C NMR spectrum suggested a β-carboline [δ 148.2 (C-1) to δ 136.0 (C-9a)]. ^1H and ^{13}C NMR spectra displayed some similarity with those of manzamine B.[2b] Two sets of $(CH_2)_2CH{=}CH(CH_2)_4N$ units [δ_C 40.0, δ_H 1.31, 2.51 (C-13) to δ_C 53.4, δ_H 2.86, 3.52 (C-20) and δ_C 34.1, δ_H 1.15, 2.00 (C-35) to δ_C 48.4, δ_H 2.74, 3.42 (C-28)]

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(2) (a) Sakai, R.; Higa, T.; Jefford, C. W.; Bernardinelli, G. *J. Am. Chem. Soc.* **1986**, *108*, 6404–6405. (b) Sakai, R.; Kohmoto, S.; Higa, T.; Jefford, C. W.; Bernardinelli, G. *Tetrahedron Lett.* **1987**, *28*, 5493–5496. (c) Ichiba, T.; Sakai, R.; Kohmoto, S.; Saucy, G.; Higa, T. *Tetrahedron Lett.* **1988**, *29*, 3083–3086.

(3) (a) Ichiba, T.; Corgiat, J. A.; Scheuer, P. J.; Kelly-Borges, M. *J. Nat. Prod.* **1994**, *57*, 168–170. (b) Crews, P.; Cheng, X.-C.; Adamczeski, M.; Rodriguez, J.; Jaspars, M.; Schmitz, F. J.; Traeger, S. C.; Pordesimo, E. O. *Tetrahedron* **1994**, *50*, 13567–13574.

(4) Baldwin, J. E.; Claridge, T. D. W.; Heupel, F. A.; Whitehead, R. C. *Tetrahedron Lett.* **1994**, *35*, 7829–7832.

(5) IR (CCl_4): ν_{max} 3380, 3150, 2990, 2900, 2840, 1645, 1620, 1450, 1440, 1320, 1235, 1150 cm^{-1}; FAB MS *m/z* 1101 ([MH − H_2O]$^+$), 561, 553. The name is coined from *kaulua*, a double-hulled voyaging canoe in Hawaiian, and thus reflects the dimeric nature of the compound.

(6) The sponge was collected on Oct 3, 1992, at a depth of 120 ft in Manado Bay, Sulawesi, Indonesia. The sponge forms large tubes, occasionally basally coalescent, each with broad apex and wide apical oscule, growing erect from muddy sand. The surface is rough and cavernous, the texture very fragile and crumbly. The color in life is brownish orange externally, orange internally, and is brown in alcohol. The skeleton is composed of loose plumose single and fasciculated tracts of strongyles between which are large spaces and abundant spicules. The sample is an undescribed species of *Prianos* (Family Desmacidonidae (?), order Poecilosclerida), the family assignment of which is uncertain. A voucher specimen has been deposited at the Natural History Museum, London, U.K. (BMNH 1992:10:3:1).

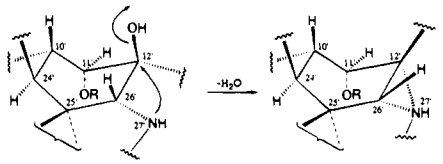
(7) Crystalline salts of **1** and its derivative were not obtained, owing to decomposition in acid, such as HCl and MTPA.

(8) Molecular ions of both **1** and **2** were not observed by FABMS (positive or negative modes using several matrices) or ESI-MS. Elementary analysis of **1** failed to confirm the molecular formula.

(9) The INADEQUATE experiment of **1** (100 mg in 0.3 mL of acetone-d_6 or $CDCl_3$) was attempted using a microprobe but gave no correlations because the ^{13}C signals were too broad.

(10) Acetylation of **1** with pyridine and acetic anhydride (for 30 min at room temperature) afforded a complex mixture, from which only monoacetate **2** was isolated by HSCCC in 53% yield.

(11) This antipodal stereochemistry at C-12′ places NH-27′ and 12′-OH in an *anti* relationship, which is believed to cause aziridine formation leading to a loss of H_2O under the MS conditions.



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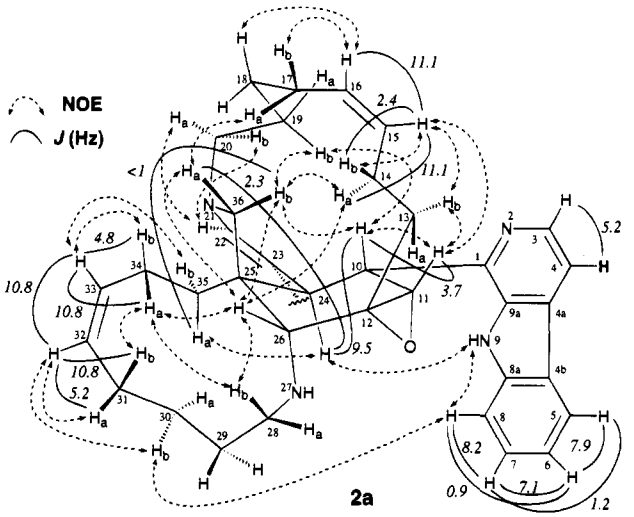
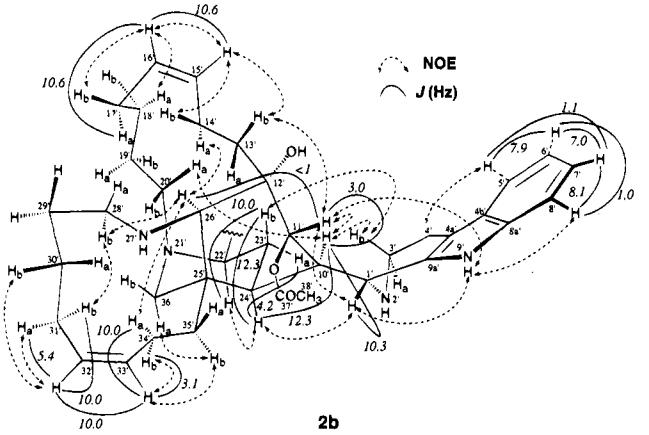
were deduced from the HOHAHA and HMQC-HOHAHA spectra. The *Z*-geometry of $\Delta^{15,16}$ and $\Delta^{32,33}$ was determined by coupling constants (11.1 and 10.8 Hz, respectively) between olefinic protons. The $^1J_{CH}$ value ($CDCl_3$, 179 Hz of C-11 for **1**) suggested the presence of an epoxide. The epoxide proton (H-11, δ 3.34) showed DQFCOSY correlation to H-10 (δ 3.50), which was coupled to H-24 (δ 2.44), and showed HMBC correlation to C-12 (δ 63.1). On the basis of the following HMBC data, the entire bicyclic octahydroisoquinoline moiety (C-10−12,26,25,36, N-21, C-22−24) was elucidated: H-26 (δ 3.08) to C-11, C-24 (δ 36.2), C-25 (δ 39.3), and C-36 (δ 49.9, vicinal to N); H-24 to C-22 (δ 129.7), C-23 (δ 113.4), C-25, and C-36; H-22 (δ 5.63) to C-36; and H-36b (δ 3.03) to C-26 (δ 60.5, vicinal to N). Additional HMBC correlations completed the planar structure of unit A ($C_{36}H_{43}N_4O$) as a 23-substituted 22,23-dehydromanzamine B: H-11 to C-13, H_2-13 to C-12; H_2-36 to C-35; H-35a to C-26; H-26 to C-28; and H-10 to C-1, 9a. Relative stereochemistry (**2a**) was determined by a NOESY experiment and H−H coupling constants. This was further supported by the similarity of ^{13}C chemical shifts of **1** and manzamine B, whose structure was established by X-ray crystallography.[2b]



Unit B. Interpretation of 2D NMR spectra inferred the presence of a 1′,2′,3′,4′-tetrahydro-β-carboline [δ 57.2 (C-1′) to δ 135.2 (C-9a′)] and two sets of $(CH_2)_2CH{=}CH(CH_2)_4N$ units [δ_C 31.2, δ_H 1.02, 1.87 (C-13′) to δ_C 46.7, δ_H 1.78, 2.42 (C-20′) and δ_C 32.8, δ_H 1.36, 1.61 (C-35′) to δ_C 48.6, δ_H 2.71, 3.44 (C-28′)]. The bicyclic moiety [δ_C 36.7, δ_H 1.47 (C-10′) to δ_C 40.0, δ_H 1.23 (C-24′)] was also deduced by NMR analyses. HMBC cross peaks [H-13′b (δ 1.02)/C-11′ (δ 80.1), C-26′ (δ 68.5); H_2-13′/C-12′ (δ 65.8, vicinal to O); H-26′ (δ 2.88)/C-28′, C-35′; H-20′b (δ 2.42)/C-36′ (δ 54.7); H-36′b (δ 1.99)/C-20′; H-10′/C-1′] completed the planar structure of unit B ($C_{38}H_{53}N_4O_3$). The *Z*-geometry of $\Delta^{15',16'}$ and $\Delta^{32',33'}$ was determined from coupling constants (10.6 and 10.0 Hz, respectively) between olefinic protons. Coupling constants [10.0 Hz for H-22′ (δ 1.84)/H-23′b (δ 1.15), 12.3 Hz for H-24′ (δ 1.23)/H-23′b and H-10′ (δ 1.47), 10.3 Hz for H-10′/H-1′ (δ 2.12), 4.2 Hz for H-23′a (δ 1.07)/H-24′, and 3.0 Hz for H-10′/H-11′ (δ 4.76)] and NOE [H-24′/H-1′,22′; H-3′b (δ 2.44)/H-10′,23′b; H-9′ (δ 8.54)/H-1′,11′; H-11′/H-1′,10′] led to the relative stereochemistry at C-1′, -10′, -11′, -22′, and -24′ as shown in **2b**. The stereochemistry at C-25′ and 26′ was deduced from *W*-type correlations [H-36′a (δ 2.91)/C-12′ (δ 65.8) in HMBC and H-11′/H-26′ in DQFCOSY]. Finally, the stereochemistry at C-12′ was determined to be antipodal to that reported for related manzamines, because big differences were observed between the ^{13}C chemical shift at C-13′ of unit B [δ 31.2 (**2**, CD_3CN), 31.1 (**1**, $CDCl_3$), and 30.6 (**1**, acetone-d_6)] and those reported for related manzamines (approximately δ 40).[2,11]



Complete Structure. HMBC cross peaks H-22,24/C-22′ (δ 64.4) and H22′/C-23 suggested that C-23 of unit A was attached to C-22′ of unit B. The FABMS fragment ion at *m/z* 561 ($C_{37}H_{45}N_4O$), corresponding to unit A + CH_2, and NOE (H-22/H-22′, H_2-23′) support this structure.

Kauluamine (**1**) may be formed from two molecules of manzamine by the following mechanism: (i) formation of unit B from unit A (reduction of C-3,4 followed by opening of the epoxide to generate a more stable cation at C-12 and addition of water to yield diol), (ii) formation of the $\Delta^{21',22'}$ enaminium ion in unit B, and (iii) addition of the unit A enamine to the enaminium ion of B (C-23 to C-22′). This mechanism is supported by the relative stereochemistry at C-12′.

Kauluamine (**1**) showed moderate immunosuppressive activity (MLR IC_{50} 1.57 μg/mL, LcV IC_{50} >25.0 μg/mL, LcV/MLR >16) in the mixed lymphoma reaction and was inactive in cytotoxicity and antiviral assays. Further bioassays are pending.

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Supporting Information Available: Table 1 (NMR data for **1**) and Table 2 (NMR data for **2**), ^1H NMR spectra of **1** and **2**, ^{13}C NMR spectra of **1** and **2**, COSY spectrum of **2**, HOHAHA spectrum of **2**, HC-COSY spectrum of **2**, HMQC spectrum of **2**, HMBC spectrum of **2**, and HMQC-HOHAHA spectrum of **2** (16 pages). This material is contained in many libraries on microfiche, immediately follows this article in the microfilm version of the journal, can be ordered from the ACS, and can be downloaded from the Internet; see any current masthead page for ordering information and Internet access instructions.

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